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Ethanolic Extract of Allophylus edulis Leaves Attenuates Gentamicin-Induced Acute Nephritis in Mice

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HIGHLIGHTS

- *Allophylus edulis* ethanol extract was tested in gentamicin-induced nephritis in mice
- Serum creatinine and urea levels elevated by gentamicin were prevented by the extract
- Creatinine and urea in urine showed lower levels in mice treated compared to gentamicin treated group
- *Allophylus edulis* showed a nephroprotective effect.

Abstract: The kidneys are organs of vital importance for the organism since they oversee maintaining the hydro-electrolytic balance and therefore the homeostasis of the organism. Therefore, damage to this organ, such as acute kidney injury, endangers people's lives, can become a long-term problem, and represent an excessive spending for health systems Medicinal plants, among them *Allophylus edulis*, are used in folk medicine to treat kidney and liver conditions. This research aimed to evaluate the activity of the ethanolic extract of *Allophylus edulis* (Sapindaceae), on gentamicin-induced nephritis in mice. The experiments were carried out in female mice, 135 mg/kg (i.p.) of gentamicin was used to induce kidney damage. Silymarin was used as a control drug for renal protection. Ethanolic extract of *A. edulis* was administered *per os*, at 50, 100, 200 and 400 mg/kg. To estimate renal functionality, creatinine, urea, and uric acid were determined in serum. Also, in urine were determined creatinine, urea, and uric acid, besides sodium, potassium, and chloride. *A. edulis* induced a reduction in the serum levels of creatinine, urea, and uric acid ($p<0.05$) in mice with renal damage induced by gentamicin when these animals were previously treated with 50 mg/kg of the extract. The reduction was significant compared to the pathological group, and these values were close to the group treated with silymarin. Also, creatinine and urea in urine of mice treated with the extract showed lower level

than gentamicin treated group. Therefore, in the model used, the ethanolic extract of *Allophylus edulis* showed a nephroprotective effect.

Keywords: *Allophylus edulis*, acute nephritis, gentamicin, mice, silymarin, creatinine.

INTRODUCTION

The kidneys are highly vascularized paired organs responsible for maintaining the homeostasis of the organism through the regulation of the volume and composition of the extracellular fluid, acid-base balance, conservation of electrolytes, metabolites, and elimination of certain waste products and foreign substances [1]. When kidney detoxification and excretion do not function, nephrotoxicity occurs. This can be due to exogenous or endogenous toxicants [2]. In acute kidney injury, there is a sudden decrease in glomerular filtration rate and consequently in renal function, both excretory and hormonal [3] This can lead to short- and long-term adverse situations in patients. A brief episode of acute kidney injury can contribute to permanent organ dysfunction such as chronic kidney disease (CKD), and therefore, to greater morbidity and mortality in these patients [4].

CKD currently affects around 850 million people worldwide, with one in ten adults suffering from it, and is a major cause of catastrophic health costs [5, 6]. Dialysis and transplantation consume 2-3% of healthcare budget in high-income countries [7]. In low- and middle-income countries like Paraguay, most people with kidney failure have insufficient access to dialysis and kidney transplantation [8]. Moreover, patients with CKD have greater prevalence of heart failure risk factors. Crucially, preventing kidney injury is important and progression to end-stage kidney disease can be delayed [9].

Nephroprotective agents have potential to minimize the effects of nephrotoxic agents. Several studies proved the effect of medicinal plants as nephroprotective [10, 11]. Medicinal plants have curative properties due to the presence of various complex chemical substances [12].

Allophylus edulis, locally named “Kokũ”, is a medicinal plant used as refresher, digestant, and, to a lesser extent, in the treatment of hepatitis and kidney disorders [13]. Previously, we have demonstrated that the ethanolic extract of *A. edulis* was able to protect the liver from acute damage induced by paracetamol in mice [14]. Nowadays, scientific information on its effect on kidney diseases is scarce. In this paper, we report the evaluation of the effect of *A. edulis* extract on acute nephritis induced by gentamicin in mice. The nephroprotective effect was evaluated by determining serum and urine levels of creatinine, urea, and uric acid. In urine, sodium and potassium levels were also measured.

MATERIAL AND METHODS

Plant material

Allophylus edulis Radlk. (A. St.-Hil., A. Juss. & Cambess.) Hieron. ex Niederl., known as “Kokũ” (Sapindaceae) leaves were collected from J.A. Saldívar, Central, Paraguay (25º26´50,4¨S y 57º 27´07,1W), identified, filed a voucher specimen in FCQ Herbarium (G Delmás 284), and extracted as described previously [14]. Briefly, the powder was extracted with previously distilled ethanol by sonicating the mixture and subsequently submitted to reflux. The solvent was evaporated, and the extract was kept in a desiccator, and freshly dissolved in ethanol/propylene glycol/distilled water (0.5:4:5.5) before oral administration in mice.

Drugs and equipment

Silymarin (Sigma Chemical Company Mo.), Gentamicin (Larjan, Veinfar, Argentina), and sodium pentobarbital from Abbott (Japan) were used; ethanol was purchased locally and distilled before use. Kits for the estimation of parameter of kidney functionality, were purchased from Wiener Lab reagent. Electrolytes determinations were done with Wash, Cal 2, Cal 3, Eschweiler. Diluent for determination of urinary electrolytes: Urine Diluent, Eschweiler. Electrolytes were determined in Eschweiler Combi-line, (Kiel, Germany). Microcentrifuge, Hermle Z216M. Metabolic cages, Suzhou Fengshi Laboratory Animal Equipment Co. Ltd. Autoanalyzer CB350i, Wiener Lab. (Rome, Italy).

Experimental animals and ethical issues

Swiss albino female mice, weighing 25-35 g, procured from the animal facility of the Facultad de Ciencias Químicas, kept in standard laboratory conditions (22±2°C, humidity of up to 60%, 12/12light/darkness). were used. They were fed daily with standard animal pellets (7 g/day each) and water *ad libitum*. For animal handling, the standards established in the Ethics Commission of the European Community were followed

[15]. The research protocol was approved by the Bioethical Committee of the Facultad de Ciencias Químicas (CEI 941/22). The animals were used only once, euthanasia was done by cervical dislocation. For their final disposal they were delivered to a company specialized in biological waste management.

Gentamicin-induced nephrotoxicity and treatments

Mice were divided into 7 groups (n=6) and treated during 9 days as follows: control (Veh; water, per os); gentamicin (Gent; water 0.1mL/10g body weight, *per os*, and gentamicin); silymarin treated group (Sil; 150 mg/kg body weight, silymarin, *per os*, and gentamicin); Ae 50, Ae 100, Ae 200 (received 50, 100, 200, and 400 mg/Kg of *A. edulis* ethanol extract, respectively, *per os*, and gentamicin. Except in control group, mice received gentamicin (135mg/kg; intraperitoneal) one hour after the detailed treatments. At the end of the treatment period, urine samples were collected after mice were transferred to individual metabolic cages during 24 h [16].

After being removed from the metabolic cages, exsanguination of mice by cardiac puncture under anesthesia were achieved, and serum was obtained. Urea, creatinine, and uric acid (mg/dL), as well as potassium and sodium (mEq/L) levels were determined. From urine sample, creatinine, urea, and uric acid (mg/kg/24h), together with sodium and potassium (mEq/kg/24h) were determined.

Data analysis

The results correspond to mean ± standard deviation (SD), $p < 0.05$ was considered statistically significant. One-way ANOVA, and Tukey`s test was done using the GraphPad Prism 8.0.1 (GraphPad Software, Inc., CA).

RESULTS

The potential nephroprotective effect of the ethanol extract of *Allophylus edulis* was evaluated in mice. The results indicated that in the animals treated with 50 mg/Kg of the extract, the serum creatinine level decreased, so that there was a significant difference with the pathological group (Gent vs Ae50, $p<0.05$, Figure 1). Likewise, the group treated with gentamicin presented a statistically significant difference with respect to the control group, indicating that acute renal damage was established by the administration of gentamicin. There was also a significant difference between the Gent and Sil groups (treated with silymarin, $p<0.05$), thus evidencing the capacity of silymarin as a protector against the damage induced to this organ. Additionally, the group treated with silymarin did not present a difference with respect to the control group, and neither was it different from the Ae 50 group. The latter could mean that the extract protects the kidney to the same extent as silymarin.

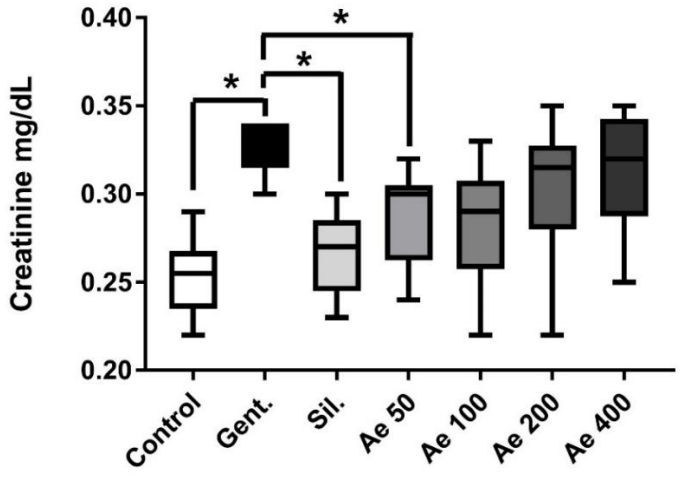

Figure 1. Creatinine level of animals treated with the ethanolic extract of *Allophylus edulis*. Each box corresponds to the mean ± deviation (n=6). One-way ANOVA, Tukey's posttest. * $p<0.05$.

Regarding serum urea levels (Figure 2), gentamicin-induced an elevation in the pathological group, statistically significant with respect to the control group ($p<0.01$) and with the silymarin group ($p<0.05$). The extract (50 mg/kg) was able to prevent urea elevation, which was evidenced by the difference observed between the Gent and Ae 50 groups. Furthermore, there was no difference between the group treated with silymarin and the control group. These results agree with what is expected from the model of nephrotoxicity

caused by gentamicin, where the glomerular filtration rate is functionally altered and the serum creatinine and urea levels rise, which are indicators of renal injury [17, 18]. Additionally, regarding the level of urea, the silymarin group did not present a significant difference with respect to the group treated with 50 mg/Kg of the *A. edulis* extract, which can be interpreted as the extract and silymarin having the same nephroprotective efficacy.

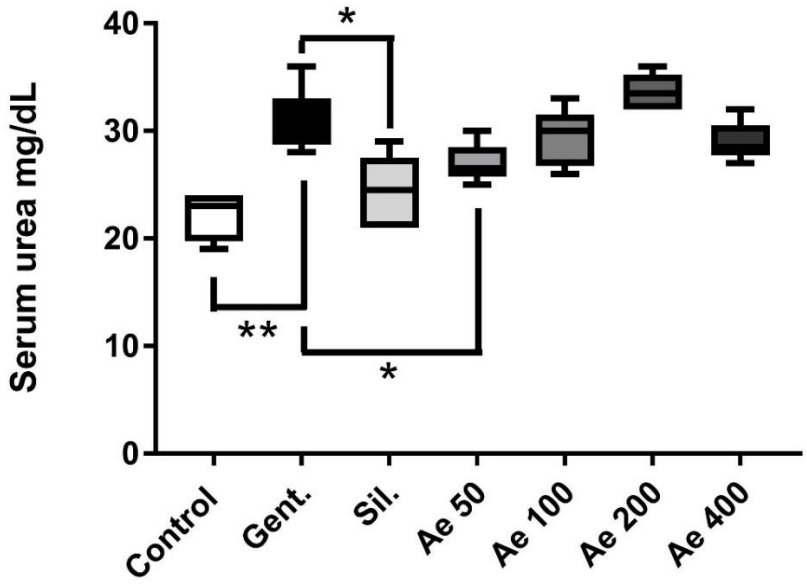

Figure 2. Urea level of animals treated with the ethanolic extract of *Allophylus edulis*. Each box corresponds to the mean ± deviation (n=6). One-way ANOVA, Tukey's posttest. *p<0.05.

Serum uric acid levels showed that the Gent pathological group was statistically different from the control group (Figure 3, p<0.05) and the silymarin group (p<0.05). In addition, the silymarin group did not present a statistically significant difference with respect to the control group, which indicates that the methodology is valid for carrying out this type of experiment. On the other hand, the pathological group presented a statistically significant difference with respect to the group treated with 50 mg/kg (Ae 50, p<0.05).

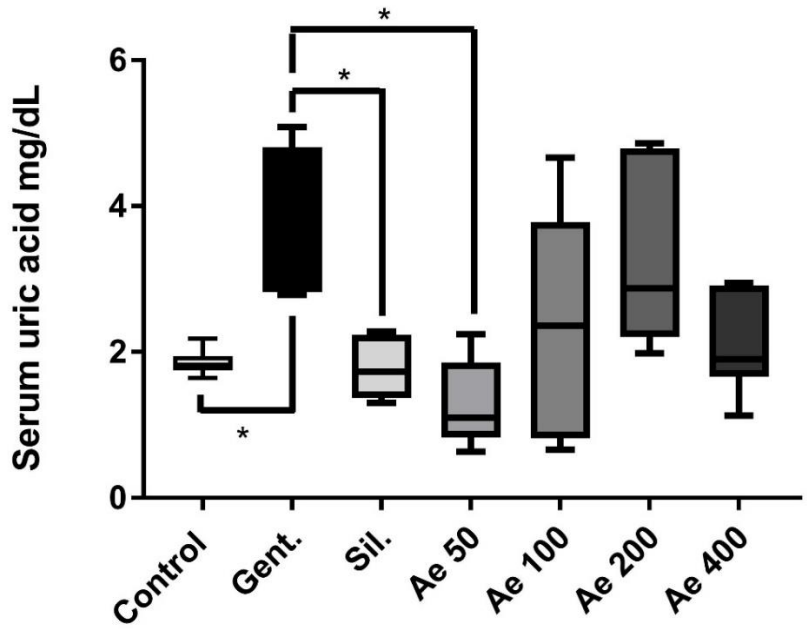

Figure 3. Uric acid level of animals treated with the ethanolic extract of *Allophylus edulis*. Each box corresponds to the mean ± deviation (n=6). One-way ANOVA, Tukey's posttest. * p<0.05.

The results in urine showed (Table 1) that in the pathological group (Gent), creatinine excretion was statistically higher than in the control group, and it was also verified that the extract statistically reduced

(p<0.05) the level of this parameter when the animals were treated with 50, 100, and 400 mg/kg of *A. edulis*, and with silymarin (p<0.01). Urinary urea of animals treated with silymarin and 50, 100 and 400 mg/kg of *A. edulis* was also significantly lower when compared to the pathological group (p<0.05). The level of uric acid in urine did not show alteration in any group.

Regarding electrolytes, in the group induced renal damage with gentamicin (Gent), there was an elevation in the excretion of sodium and chloride ions, significantly different from the control group, and there were no changes in the animals treated with the extract.

Table 1. Effect of oral administration of the ethanolic extract of *Allophylus edulis* on renal profile parameters and 24-hour urine electrolytes.

Parameter	Groups						
	Control	Gent	Silymarin	Ae 50	Ae 100	Ae 200	Ae 400
Creatinine[#]	14,98±3,58*	30,47±5,81	19,37±4,24**	18,27±2,19*	18,64±4,39*	20,47±3,09	19,55±3,61*
Urea[#]	0,84±0,26	1,31±0,16	0,78±0,14**	0,85±0,14*	0,83±0,17*	0,92±0,17	0,85±0,16*
Uric acid[#]	1,88±0,88	4,61±1,44	2,63±0,29	3,13±0,39	2,55±0,49	3,84±0,43	3,03±0,54
Na^{+} [##]	1,92±0,57*	4,83±1,51	2,92±0,63*	4,10±0,69	5,28±1,34	5,10±1,27	5,31±1,79
K^{+}[##]	3,02±1,16	3,66±0,43	4,36±0,91	2,86±0,45	3,77±0,73	6,53±1,18*	5,93±1,01*
Cl^{-} [##]	3,66±1,51*	9,58±2,50	5,95±1,12	7,04±1,16	7,17±1,80	6,60±1,00	6,59±1,75

[#] mg/kg/24h; [##] mEq/kg/24h. The data correspond to the mean ± standard deviation of each group (n=6). One-way ANOVA, Tukey's posttest. *p<0.05, **p<0.01, significantly different from the pathological group.

DISCUSSION

Gentamicin, the most often utilized aminoglycoside due to its low cost, was used as nephrotoxic. It acts on mitochondria and promotes the formation of reactive oxygen species. Gentamicin induces renal damage by selective accumulation in the renal proximal tubular cells, causing glomerular deterioration, cellular necrosis, and fibrosis, as well as inflammation [19]. This drug induces increased serum creatinine and urea levels [20]. It has been shown that both, creatinine, and urea levels also increase in urine [21].

These two main markers of renal damage elevated by gentamicin, have shown reduced levels with the concomitant administration of *A. edulis* extract. In serum, although with all doses tested lower doses were evidenced, only with 50 mg/kg the difference was statistically significant. In urine, the same result is reached with 50 mg/kg, 100 mg/kg, and 400 mg/kg. These results indicated that this plant could protect the kidney from damage induced by gentamicin.

Many natural products or natural products derivatives have demonstrated to protect against nephrotoxicity or oxidative stress [22, 23]. Nephroprotective actions of natural products are generally mediated by their antioxidant properties, and to some extent, anti-inflammatory functions. The nephroprotective effect of curcumin is mainly mediated by its strong antioxidant properties as well as through mitochondrial function maintenance [24]. Stevioside was found to exert its nephroprotective activity in cisplatin-induced nephrotoxicity by suppressing oxidative stress, inflammation, and apoptosis [25]. Quercetin was found to display its protection through its antioxidant effect among other mechanisms [26].

Oral administration of *A. edulis* extract was previously reported to be safe and to have hepatoprotective activity, both *in vitro* and *in vivo*. In addition, a great antioxidant activity was reported in agreement with a high total phenolic content [27, 14]. Moreover, quercetin, and many other phenolic compounds have been previously reported in this plant [27, 28]. The mechanism could be mediated by their antioxidant properties. The ameliorative effect of *A. edulis* was showed, however, more studies are required to understand mechanistic pathways on gentamicin-induced nephrotoxicity, to analyze effects on tissues and other markers of kidney damage (proteinuria, blood urea nitrogen, markers of glomerular filtration rate and tubular dysfunction, among others) and identify bioactive compounds.

CONCLUSION

Allophylus edulis extract demonstrated ameliorative effect in gentamicin-induced nephrotoxicity assay in mice, so a possible nephroprotective activity is inferred. This is evidenced by the reduced level of serum and urinary creatinine and urea in mice. These results are consistent with traditional use of this plant to treat kidney diseases. More studies are required to understand mechanistic pathways, and to identify bioactive compounds.

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